

**Utility Application**

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APPLICATION FOR U.S. LETTERS PATENT

Title:

MICROFUIDIC DEVICE

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## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This is a continuation-in-part application of U.S. Patent Application Serial Number 10/621,868, filed July 17, 2003, which is a continuation of U.S. Patent Application Serial Number 09/811,741, filed March 19, 2001; and is a continuation-in-part of U.S. Patent Application Serial Number 09/674,457, filed January 5, 2001, which claims priority to PCT Application Number PCT/IB99/00907, international filing date May 7, 1999, and all applications are incorporated herein by reference.

## TECHNICAL FIELD

**[0002]** The present invention relates to microfluidic devices which may be used for a variety of biological processes, e.g. screening putative biologically active molecules against cell cultures or separating biological materials, the preparation of such devices and their use.

**[0003]** The present invention also relates to a microfluidic device, which can be interfaced to a mass spectrometer (MS). The device comprises a microchannel structure having a first port (inlet port) and a second port (outlet port). A sample to be analysed is applied to the first port and presented to the mass spectrometer in the second port. This second port will be called an MS-port. There may be additional inlet and outlet ports. During passage through the microchannel structure the sample is prepared to make it suitable for analysis by mass spectrometry.

**[0004]** The sample presented in an MS-port will be called an MS-sample. An analyte in an MS-sample is an MS-analyte. "Sample" and "analyte" without prefix will primarily refer to a sample applied to an inlet port.

**[0005]** One important aspect of the present invention concerns mass spectrometry in which the MS-samples are subjected to Energy Desorption/Ionisation from a surface by input of energy. Generically this kind of process will be called EDI and the surface an EDI surface in the context of the invention. Typically EDIs are thermal desorption/ionisation (TDI), plasma desorption/ionisation (PDI) and various kinds of irradiation desorption/ionisation (IDI) such as by fast atom bombardment (FAB), electron impact etc. In the case a laser is used the principle is called laser desorption/ionisation (LDI). Desorption may be assisted by presenting the MS analyte together with various helper substances or functional groups on the surface. Common

names are matrix assisted laser desorption/ionisation (MALDI) including surface-enhanced laser desorption/ionisation (SELDI). For MALDI see the publications discussed under Background Publications below. For SELDI see WO 0067293 (CIPHERGEN Biosystems).

**[0006]** The surface from which desorption/ionisation is intended to take place is called an EDI surface.

**[0007]** By microformat is meant that in least a part of the microchannel structures the depth and/or width is in the microformat range, i.e.  $< 10^3 \mu\text{m}$ , preferably  $< 10^2 \mu\text{m}$ . In the most typical microformat structures either the width and/or the depth are in principle within these ranges essentially everywhere between the sample inlet port and the MS-port.

## BACKGROUND OF THE INVENTION

**[0008]** PCT patent application 97/21090 describes a microanalytical/microsynthetic system for biological and chemical analysis which comprises a rotatable microplatform, for example a disk, having inlet ports, to microchannels, detection chambers and outlet ports through which fluid may flow.

**[0009]** It has now been found that microfluidic devices can be prepared in which fluid flow may be controlled by having different surfaces of the substrate forming the device having different surface characteristics. By "microfluidic devices" is meant devices that can handle microvolumes of reagents, for example samples of less than  $1 \mu\text{l}$ , suitably less than  $500 \text{ nl}$  and preferably between  $1$  and  $10 \text{ nl}$ , may be introduced into the device. By "fluid" is meant dry powders and liquids, including suspensions of particulates in liquids.

**[0010]** Accordingly, in a first aspect the present invention provides a microfluidic device adapted such that the flow of fluids within the device is controlled by different surfaces of the device having different surface characteristics.

**[0011]** The nature of the surface characteristics which control fluid flow is dependent upon the nature of the fluid itself. For example, when the fluid is a liquid, the surface characteristic that controls the flow of the liquid is preferably the surface energy of the material, e.g. low energy surfaces are normally hydrophobic whilst high energy surfaces are normally hydrophilic.

**[0012]** The energy of a surface may be measured in terms of the critical surface tension(see for example Surface and Interfacial Aspects of Biomedical Polymers, Vol I, Plenum Press, New York, 1985, Ch.7). When the fluid is particulate, the surface characteristic that controls the flow of the particles is dependent upon the nature of the particles, e.g. the surface is treated to interact with the particle, for example if the particle carries a charge the surface will have the same or opposite charge, similarly if the particle is magnetic the surface may be permanently or transiently magnetised.

**[0013]** For some time there has been a demand for microfluidic sample handling and preparation devices with integrated MS-ports. This kind of devices would facilitate automation and parallel experiments, reduce loss of analyte, increase reproducibility and speed etc.

**[0014]** WO 9704297 (Karger et al) describes a microfluidic device that has an outlet port that is claimed useful when conducting electrospray ionisation mass spectrometry (ESI MS), atmospheric pressure chemical ionisation mass spectrometry (APCI MS), matrix assisted laser desorption/ionisation mass spectrometry (MALDI MS) and a number of other analytical principles.

**[0015]** US 6,110,343 (Ramsey et al) describes an electrospray interface between a microfluidic device and a mass spectrometer.

**[0016]** US 5,969,353 (Hsieh) describes an improved interface for electrospray ionization mass spectrometry. The interface is in the form of an electrospray tip connected to a microchannel structure of a chip.

**[0017]** US 5,197,185 (Yeung et al) describes a laser-induced vaporisation and ionization interface for directly coupling a microscale liquid based separation process to a mass spectrometer. A light-adsorbing component may be included in the eluting liquid in order to facilitate vaporisation.

**[0018]** US 5,705,813 (Apffel et al) and US 5,716,825 (Hancock et al) describe a microfluidic chip containing an interface between a microfluidic device and an MALDI-TOF MS apparatus. The microfluidic device comprises

**[0019]** (a) an open ionisation surface that may be used as the probe surface in the vacuum gate of an MALDI-TOF MS apparatus (column 6, lines 53-58 of US 5,705,813) or

**[0020]** (b) a pure capture/reaction surface from which the MS-analyte can be transferred to a proper probe surface for MALDI-TOF MS (column 12, lines 13-34, of US 5,716,825).

**[0021]** These publications suggest that means, such as electrical connections, pumps etc, for transporting the liquid within a microchannel structure of the device are integrated with or connected to the device. This kind of transporting means imposes an extra complexity on the design and use, which in turn may negatively influence the production costs, easiness of handling etc of these devices.

**[0022]** US 5,705,813 (Apffel et al) and US 5,716,825 (Hancock et al) are scarce about the proper fluidics around the MALDI ionisation surface, the proper crystallisation on the MALDI ionisation surface, the proper geometry of the port in relation to crystallisation, evaporation, the incident laser beam etc, the proper arrangement of conductive connections to the MALDI ionisation surface for MALDI MS analysis.

**[0023]** WO 04297 (Karger et al) and WO 0247913 (Gyros AB) suggest to have microchannel structures in radial or spoke arrangement.

**[0024]** A number of publications referring to the use of centrifugal force for moving liquids within microfluidic systems have appeared during the last years. See for instance WO 9721090 (Gamera Bioscience), WO 9807019 (Gamera Bioscience) WO 9853311 (Gamera Bioscience), WO 9955827 (Gyros AB), WO 9958245 (Gyros AB), WO 0025921 (Gyros AB), WO 0040750 (Gyros AB), WO 0056808 (Gyros AB), WO 0062042 (Gyros AB) and WO 0102737 (Gyros AB) as well as WO 0147637 (Gyros AB), WO 0154810 (Gyros AB), WO 0147638 (Gyros AB), and WO 0146465.

**[0025]** See also Zhang et al, "Microfabricated devices for capillary electrophoresis – electrospray mass spectrometry", Anal. Chem. 71 (1999) 3258-3264) and references cited therein.

**[0026]** Kido et al., ("Disc-based immunoassay microarrays", Anal. Chim. Acta 411 (2000) 1-11) has described microspot immunoassays on a compact disc (CD). The authors suggest that a CD could be used as a continuous sample collector for microbore HPLC and subsequent detection for instance by MALDI MS. In a preliminary experiment a piece of a CD

manufactured in polycarbonate was covered with gold and spotted with a mixture of peptides and MALDI matrix.

**[0027]** Another embodiment is to provide improved means and methods for transporting samples, analytes including fragments and derivatives, reagents etc in microfluidic devices that are capable of being interfaced with a mass spectrometer.

**[0028]** Another embodiment is to provide improved microfluidic methods and means for sample handling before presentation of a sample analyte as an MS-analyte. Sub-objects are to provide an efficient concentration, purification and/or transformation of a sample within the microfluidic device while maintaining a reproducible yield/recovery, and/or minimal loss of precious material.

**[0029]** Another embodiment is to provide improved microfluidic methods and means that will enable efficient and improved presentation of the MS-sample/MS-analyte. This object in particular applies to MS-samples that are presented on a surface, i.e. an EDI surface.

**[0030]** Another embodiment is to enable reproducible mass values from an MS-sample that is presented on a surface, i.e. on an EDI surface.

**[0031]** Another embodiment is to provide improved microfluidic means and methods for parallel sample treatment before presentation of the analyte to mass spectrometry. The improvements of this object refer to features such as accuracy in concentrating, in chemical transformation, in required time for individual steps and for the total treatment protocol etc. By parallel sample treatment is meant that two or more sample treatments are run in parallel, for instance more than five, such as more than 10, 50, 80, 100, 200, 300 or 400 runs. Particular important numbers of parallel samples are below or equal to the standard number of wells in microtiter plates, e.g. 96 or less, 384 or less, 1536 or less, etc

**[0032]** Another embodiment is to provide a cheap and disposable microfluidic device unit enabling parallel sample treatments and having one or more MS-ports that are adapted to a mass spectrometer.

## BRIEF SUMMARY OF THE INVENTION

**[0033]** In one embodiment there is provided a microfluidic device comprising a substrate whose surface is treated to provide areas having different surface characteristics, said areas being arranged to enable control of the flow of fluids passing across the substrate. For example, the substrate may have a hydrophobic surface interspersed with a plurality of hydrophilic areas. Alternatively, the substrate may have a hydrophilic surface interspersed with a plurality of hydrophobic areas. Preferably, the substrate is not formed from a hydrated oxide material. Preferably the substrate is formed from a plastics material such as a polycarbonate or a hydrocarbon polymer (including a halogenated hydrocarbon polymer) such as a polyolefin or a similar material which imparts a hydrophobic surface to the substrate. Whilst the substrate is formed from a material which provides a hydrophobic surface to the substrate, this hydrophobic surface can be treated, as described hereinafter, to convert it to a hydrophilic surface.

**[0034]** Preferably, the device has a second substrate approximately parallel to the first; the first, and optionally the second substrates having surface areas of different surface characteristics that control the flow of fluid within the device.

**[0035]** When the substrate comprises a hydrophobic surface interspersed with hydrophilic areas, these hydrophilic areas suitably comprise a plurality of arrays of hydrophilic spots on the hydrophobic surface. By an array of spots is meant a number of spots, suitably greater than 10 and preferably greater than 50, for example 200, which are arranged on the surface within the same fluid pathway in a predetermined pattern. The array may be single dimensional - i.e. a line of spots, or multi-dimensional.

**[0036]** By areas of different surface characteristics is meant that areas of the surfaces of the substrate have different relative characteristics, for example, in the case of liquids, different relative hydrophobicities or hydrophilicities. Boundaries between such areas may in effect form "walls" defining the flowpath of fluid within the device. Alternatively, they may form "valves" preventing the flow of fluid across the boundary until the fluid has either been provided with sufficient energy to enable it to overcome the difference in surface energies of the surfaces or, if the characteristic of the surface can be imparted to the surface transiently, e.g. in the form of an electric charge, magnetic field, particular temperature or light intensity, by changing the characteristic of the surface.

**[0037]** When a boundary between a hydrophilic and hydrophobic surface is used to create a valve, also referred to herein as a break, the physical parameters associated with the valve, or break, may be designed to give predetermined breakthrough pressures (that is to say the pressure required to make fluid pass over the boundary). Such physical parameters include the dimensions of the valve in terms of its width and breadth compared with the corresponding dimensions of the channel leading into it, the hydrophobicity of the surface forming the valve and, when the device is a rotational disk, the length of the channel leading into the valve.

**[0038]** Normally, it will be possible to pass fluid through a valve of the present invention a number of times. However, certain fluids (for example serum contains a high protein content) may modify the hydrophobic surface making this hydrophilic so that the valve only works once. In this case, when it is desired to add further fluid this will be introduced via a second channel, which also contains a hydrophobic/hydrophilic valve, which connects into the first channel.

**[0039]** It is believed that the terms hydrophobic and hydrophilic are well known to those skilled in the art. That a surface is hydrophobic means that water does not spread on it but stands up in the form of droplets the contact angle being that measured from the plane of the surface, tangent to the water surface at the three phase boundary line. Thus, hydrophobic surfaces have been characterised as having high contact angles with water, often in the range 40 to 110 degrees (Zettlemeyer, Hydrophobic Surfaces, Ed.F.M.Fowkes, Academic Press, (New York). Hydrophilic surfaces are those which have low contact angles with water, often in the range 1 to 25 degrees. However, without limitation and for the purpose of guidance only, suitable hydrophobic surfaces include hydrocarbon polymers, including halogenated hydrocarbon polymers, see for example table 1, whilst suitable hydrophilic surfaces include non-contaminated metal oxides, siliceous materials, such as glass and polysaccharides. Surfaces of materials may be modified to change their properties, i.e. hydrophilic materials may be given hydrophobic properties by surface treatment with a hydrophobic material such as hydrocarbon, perfluorinated hydrocarbon or silicone containing species. Likewise, hydrophobic materials can be made hydrophilic by the introduction of charged groups or hydroxyl, amide or polyether groups on the surface. It is often convenient to convert the whole (or substantially the whole) of a hydrophobic surface to a hydrophilic surface and to then introduce areas of hydrophobicity onto the hydrophilic surface. A small fraction of a monomolecular layer may be sufficient to change the



surface characteristics drastically. When the hydrophobic/hydrophilic boundaries form "walls" and "valves", then the surface energy difference to form a wall may be the same or different to that for a valve, however the energy difference for a wall will normally be higher than that for a valve.

**[0040]** Some or all of the areas interspersed on the surface (be they hydrophobic or hydrophilic) may suitably be treated to allow the culture of cells on them. In this embodiment the device may for example be used for screening intracellular events (see for example European Patent 650396 B on how this may be performed).

**[0041]** Suitable liquids for use in the devices of the present invention are those which have a surface tension preferably greater than  $18 \text{ mNm}^{-1}$ . Aqueous solutions or suspensions which have a surface tension greater than  $50 \text{ mNm}^{-1}$  are preferred.

**[0042]** Suitable particulates for use in the devices of the present invention are powders or beads having a particle size of less than  $200\mu\text{m}$ . Such powders or beads are preferably treated in some way, for example they to carry an electric charge or are magnetic, that makes them more amenable to flow through the device of the present invention. Whilst the present invention anticipates the use of particulates in the devices of the present invention in the absence of a liquid carrier, they may also be present in such a liquid carrier.

**[0043]** The microfluidic device is preferably circular and adapted for rotation about its axis. Such adaptation may take the form of a hole at the axis of one or both substrates which is capable of engaging a drive shaft. Other methods of rotating the device include clamping the device and contacting the perimeter with a moving surface, for example moving wheels, or placing the device on a turntable and spinning the turntable.

**[0044]** When the device is circular the fluid inlet is normally towards the axis of the device. The inlet may be a single port attached to an annular feed channel within the device or it may be a series of ports arranged at spaced angular intervals around the axis. An annular outlet is normally located towards the circumference of the device. Fluid may flow in a laminar manner across the surface of the device or it may flow in channels formed either by hydrophobic/hydrophilic boundaries or by interior walls connecting the two substrates. These interior walls are conveniently arranged radially around the axis of the device. The channels are

normally of suitable dimensions to enable capillary forces to act upon the fluid within the channel.

[0045] When the device is adapted for cell culture it is preferable to have a source of gases available which aid cell growth. In this case, there will be one or more gas inlets in the device, which are conveniently situated in close proximity to the cells to be cultivated. Gas pathways are provided connecting the gas inlets to the cells or the fluid pathways connected to the cells, enabling culture medium/nutrients and gas, e.g. air, to be supplied down the fluid pathways.

[0046] The substrates forming the device are conveniently parallel and are preferably sufficiently close together to enable liquids in the device to be subject to capillary forces, suitably less than two millimetres apart, preferably less than one millimetre. Thus a liquid can be fed into the fluid inlet and will then be sucked down the fluid pathways by capillary action until it reaches a valve conveniently a hydrophobic/hydrophilic boundary, past which it cannot flow until further energy is applied. This energy may for example be provided by the centrifugal force created by rotating the device. Once the centrifugal force is sufficient, the liquid will flow over the valve and continue in an outward direction until it reaches the annular fluid outlet. When the areas interspersed on the surface are hydrophilic, the fluid will have a surface tension greater than  $50 \text{ mNm}^{-1}$ , for example aqueous solutions or suspensions, and when they are hydrophobic the fluid will be hydrophobic, e.g. non polar organic solvents. Thus, the fluid will be attracted to the areas/spots on the surface.

[0047] In one embodiment the areas form arrays of spots of hydrophobicities or hydrophilicities of a predetermined pattern. Such arrays can be used to build up deposits of materials to be analysed e. g. antibodies, oligonucleotides or a chemical library. For example, droplets of solvents containing the material to be analysed form on the surface, the solvent evaporates and the material is deposited.

[0048] In a second embodiment pathways are formed between parallel substrates. In this case surfaces forming the fluid pathways may themselves have areas of alternating hydrophobicity and hydrophilicity forming arrays of spots as above. These alternating areas of hydrophobicity/hydrophilicity may be formed on the surface of one or both substrates, e.g. one surface may have alternating areas whilst the opposing surface does not.

**[0049]** Alternatively, the fluid pathways may contain a substance for separating chemical/biological materials, e.g. a gel for chromatography or electrophoresis or beads may be trapped in the pathways for carrying out assays; for example, scintillation proximity assays or cells can be trapped in the pathways through specific surface recognition.

**[0050]** Areas of hydrophobicity/hydrophilicity on a surface may be formed by methods well known to those skilled in the art, for example:

**1) Masking and plasma treatment**

**[0051]** This is applicable to most surfaces and enables different degrees of hydrophilicity/hydrophobicity to be achieved with ease. A mask (adhesive tape or cast film) is attached so that it fits tightly to all the surface features. Plasma treatment is then carried out on the non-masked surface.

**2) Hydrophilic "photoresist"**

**[0052]** The plastic surface is coated with a very thin layer of hydrophilic polymer (e.g. a polyvinylcinnamate) which is crosslinked by illumination through a mask. Non-crosslinked polymer is washed off.

**3) Crosslinkable surface active polymer**

**[0053]** A surface active, reactive polymer is adsorbed from aqueous solution to the plastic surfaces and illuminated through a mask. Non-crosslinked polymer is washed off.

**4) Polymerisable surfactants**

**[0054]** A monolayer of polymerisable surfactant (e.g. the diacetylene functional phospholipids from Biocompatibles Ltd) is adsorbed and illuminated through a mask. Non-crosslinked surfactant is washed off.

**5) Photo-oxidation**

**[0055]** The plastic surfaces are illuminated with a powerful light source (e.g. Hg lamp or uv laser) through a mask so that the illuminated areas are oxidised by atmospheric oxygen.

**6) Electron beam treatment**

**[0056]** The plastic is irradiated through a mask so that irradiated areas are in contact with air (or other reactive medium) and are oxidised creating hydrophilic groups.

**[0057]** The present inventors have recognized that several of the above-mentioned objects can be met in the case inertia force is used for transportation of a liquid within a microfluidic device of the kind discussed above. This is applicable to any liquid that is used in the microfluidic device, for instance washing liquids and liquids containing at least one of (a) the analyte including derivatives and fragments thereof, (b) a reagent used in the transformation of the sample/analyte, etc.

**[0058]** The present inventors have also recognized that one way of optimizing an EDI area within a microfluidic device is related to (a) the design and/or positioning of a conducting layer in the EDI area, and/or (b) the importance of a conductive connection to the EDI area for MS analysis.

**[0059]** This kind of connection supports the proper voltage and/or charge transport at the EDI area, for instance.

**[0060]** Improper conductive properties may interfere with the mass accuracy, sensitivity, resolution etc.

**[0061]** Conductive and non-conductive properties shall refer to the property of conducting electricity.

**[0062]** An aspect of the invention is a method for transforming a liquid sample containing an analyte to an MS-sample containing an MS-analyte and presenting the MS-sample to a mass spectrometer. The method is characterized in comprising the steps of: (a) applying the liquid sample to an inlet port of a covered microchannel structure of a microfluidic device, (b) transforming the liquid sample to an MS-sample containing the MS-analyte within the microchannel structure, and (c) presenting the MS-analyte to the mass spectrometer.

**[0063]** A further characteristic feature of this aspect is that transport of liquid within the microchannel structure is performed by the application of inertia force. Inertia force may be the driving force in only a part of the microchannel structure or the whole way from an inlet port to an MS-port and/or to any other outlet port. It is believed that the most general and significant advantages of using inertia force will be accomplished in so called transporting zones, i.e. between zones having predetermined functionalities, or for overcoming or passing through

valve functions within a microchannel structure (capillary junctions, hydrophobic breaks etc). See below. The MS-port typically has a conductive connection for MS analysis.

**[0064]** At the priority date the most important inertia force for microfluidic devices is centrifugal force. In other words a force that causes outward radial transportation of liquid by spinning a disc in which the liquid is located within microchannel structures that are oriented radially (spinning is around an axis that is perpendicular to the plane of the disc). Inertia force caused by other changes of direction and/or magnitude of a force can be utilized.

**[0065]** Another aspect also includes the corresponding mass spectrometric method, i.e. the same method together with the actual collection of a mass spectrum and analysis thereof, for instance in order to gain molecular weight and structure information about the analyte.

**[0066]** Another aspect is further defined as discussed below for the microfluidic device as such and for the individual steps.

**[0067]** Another aspect of the invention is a microfluidic device containing one, two or more microchannel structures containing an inlet port, an MS-port and a flow path connected to one or both of the ports. The device may be disc-formed or otherwise provide a planar form. The characteristic feature is that the microchannel structures are oriented radially in an annular/circular arrangement. Thus each microchannel structure extends in a radial direction with an inlet port at an inner position and an outlet port such as an MS-port, at an outer peripheral position. The MS-port typically has a conductive connection as discussed above. The features discussed below further define this aspect of the invention.

**[0068]** Another aspect of the invention is a microfluidic device comprising a plurality of covered microchannel structures as defined herein and with each microchannel structure having an MS-port comprising an EDI area in which there is a conducting layer (layer I). This aspect of the present invention comprises a number of subaspects having the common characteristic feature that there may be a conductive connection to layer (I) of each individual EDI area, as discussed above. There are also features that are distinct for each subaspect.

**[0069]** Another subaspect is further characterized in that layer (I) of each EDI area is part of a continuous conducting layer that is common for two or more up to all of the EDI-areas.

[0070] Another subaspect is further characterized in that in each EDI area there is a non-conducting layer (layer II) between layer (I) and the surface of the EDI area. Layer (II) in each EDI area may be part of a continuous non-conducting layer that is common for two or more up to all of the EDI-areas.

[0071] Another subaspect is further characterised in that each MS-port has an opening that is restricted by a lid which is common for and covers a number of microchannel structures. The lid may have a conducting layer that at least embraces the openings that are present in the lid. The conducting layer may be continuous in the sense that it covers at least the areas around and between the openings of two or more up to all of the MS-ports. This layer may have a conductive connection as discussed above.

[0072] Another subaspect is similar to the third subaspect in the sense that there is a lid covering at least a part of each microchannel structures. In this subaspect the lid also covers or restricts the openings of the MS-ports and is removable to an extent that enables exposure of the opening in each MS-port, for instance exposing the surfaces of EDI areas. For EDI ports the removal will facilitate irradiation and the desorption/ionisation of the MS-analyte. The removal may also facilitate evaporation of volatile components.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0073] In order that the invention may be better understood, several embodiments thereof will now be described by way of example only and with reference to the accompanying drawings in which:

[0074] Figure 1 is a diagram of a surface treated in accordance with the invention;

[0075] Figures 2 and 3 are diagrams similar to Figure 1, showing different arrangements;

[0076] Figure 4 is a diagram of a twin substrate microfluidic device according to the invention;

[0077] Figure 5 is a diagram to illustrate the use of hydrophilic areas to grow cells;

**[0078]** Figure 6 is a partial plan view of a rotary disc microfluidic device according to the invention; and

**[0079]** Figure 7 is a view of part of Figure 5, illustrated in greater detail;

**[0080]** Figure 8 shows a section of a CD having two consecutive inner annular hydrophilic channels.

**[0081]** Figure 9-11 illustrate various microchannel structures that have an MS-port.

**[0082]** Figure 12 illustrates an MS-port in form of an electrospray (sideview).

**[0083]** Figures 13a-f illustrate various design and positions of the conducting layer (I) in MS-ports containing an EDI area (cross-sectional sideview of two ms ports). The microfluidic device is fabricated in a planar substrate.

**[0084]** Figure 14 illustrates an arrangement around EDI MS-ports with layer (I) and conductive connections (transparent lid, seen from above).

**[0085]** Figures 15a-b illustrate a variant of an EDI-port with a transparent lid (seen from above and in a cross-sectional sideview, respectively).

#### DETAILED DESCRIPTION OF THE INVENTION

**[0086]** Referring firstly to Figure 1, there is shown a mask with an array of 6x6 hydrophilic spots 1, each of 3x3 mm on a 50x50 mm hydrophobic surface 2, which was made in Mac DrawPro and printed on a laser printer. The printout was copied on to a transparency sheet in a copying machine.

**[0087]** The volume of a 25 mm thick film on a 50x50 mm surface 2 is 62.5 ml. This volume polyacrylamid (PAA) was deposited on the hydrophobic side of a Gelbondä film and the above mask was placed on top of the droplet. The area under the mask was wetted by capillary forces (a small portion of the solution did end up outside the mask). Photopolymerisation through the mask was carried out for 3 minutes exposure time. The mask was removed and the surface was rinsed with water. A clear pattern was visible due to the selective wetting at the PAA surface.

[0088] Figure 2 illustrates a disc substrate 3 having a hydrophobic surface on which are formed eight 6x5 arrays of hydrophilic spots 1. Figure 3 illustrates a one-dimensional array of hydrophilic spots 1 on a hydrophobic surface 4. As will be explained, with a suitable force applied, a fluid can be caused to pass from spot to spot so that the structure forms a defined channel for fluid flow.

[0089] Figure 4 illustrates an arrangement comprising top and bottom plates 5,6 in the form of rotatable discs, having a common axis of rotation. The discs are illustrated far apart, for the purpose of clarity; in practice, the discs will be spaced apart by a distance defined by annular supporting walls 7 which distance will be suitable for the movement of liquid between the plates by capillary action.

[0090] The top disc 5 is provided with inlet holes 8 for supplying liquids to the interior. Lining up with these are corresponding areas 9 on the upper surface of the bottom disc 6, which are hydrophilic. Passing in an axial direction between the areas 9 is an elongate area 10, which is also hydrophilic. The remaining parts of the upper surface of disc 6 are hydrophobic. The elongate area 10 effectively forms a channel for liquid between the areas 9. The hydrophilic surface of area 10, bounded on both sides by the hydrophobic upper surface of disc 6 ensures that the liquid pathway is clearly defined by the "walls" which are formed by the interface between the hydrophobic and hydrophilic areas.

[0091] If the discs are rotated together about their common axis, it will be seen that centrifugal force will push liquid along the channel formed by area 10 from the innermost area 9 to the outermost area 9.

[0092] Figure 5 illustrates how cells might be applied to a hydrophilic area 2. An inlet 23 is provided for introduction of cells and reagent and a hydrophobic channel 24 is provided for respiration of the cells during their growth on the area 2 and for rinsing between tests.

[0093] Reference is now made to Figures 6 and 7 which show a microfluidic device in the form of a compact disc (CD) 10 on which are formed hydrophobic and hydrophilic areas to enable liquids to be directed about the surface of the disc to enable the automatic and simultaneous carrying out of multiple chemical/biological tests on multiple samples.



[0094] Figure 6 shows a section of the compact disc 10, having a perimeter edge 11, and central hole 12 about which it may be mounted for rotation to within a compact disc reader (not shown). On the surface of the compact disc are formed 40 sector-shaped multi-dimensional arrays 16 of hydrophilic spots. As is made clear in the enlarged view A in Figure 7, the spots are arranged in individual straight channels 13 radiating radially from the centre of the disc. Each channel comprises alternate hydrophobic areas or breaks 14 and hydrophilic areas or spots 15. The hydrophobic breaks 14 are typically 75  $\mu\text{m}$  wide in the radial direction. The hydrophilic spots 15 are typically 108  $\mu\text{m}$  wide in the radial direction.

[0095] In the illustrated embodiment, there are 20 channels in each array 16 and there are 200 hydrophilic spots 15 in each channel. Thus, each array 16 contains 4000 hydrophilic spots.

[0096] The channels in each array 16 begin in a common hydrophilic area 17 and end in a common hydrophobic area 18, constituting a break. Positioned radially outwards from the hydrophobic area 18 is a common waste channel 19.

[0097] Liquid reagent for use in carrying out the tests is introduced into an inner annular channel 20 which is common to all of the arrays 16. Extending from the channel 20 are 40 radially extending hydrophobic breaks 21, each extending to the hydrophilic area 17 of a respective array 16. A sample to be tested is introduced into the hydrophilic area 16 at 22. In this way, 40 different samples can be tested simultaneously.

[0098] Sample testing is carried out by applying to each of the hydrophilic areas 14 a sample of a known reactant, for example a known oligonucleotide. It will be seen that the device has the potential for testing each sample against 4000 different reactants. A cap may be formed on each hydrophilic spot by evaporation and accurate pre-concentration will occur on vaporisation.

[0099] Next the reagent channel 20 is filled and the disc is spun to cause the reagent to jump across the "valve" caused by the hydrophobic break 21 and radially outwardly to the waste channel 19. Progress along the individual channels 13 is by a series of jumps across the effective "valves" caused by the hydrophilic breaks 14. The force required to overcome the breaks is provided by the centrifugal action of the spinning disc.

**[0100]** Once the reagent is issuing into the waste channel 19 the disc is stopped and liquid sample added at 22. Typically the sample volume is 0.1  $\mu$ l. The disc is now spun at 2 alternating speeds (for hybridisation mixing) whereupon the centrifugal force will move the liquid plug out along channels 13, and capillary action will move the liquid back up. Typically, the sample volume required for each spot 15 is 44 pl.

**[0101]** Reading of the test results is carried out by examining the individual spots 15 using a suitable reader. After the test is completed, the disc may be rinsed by the application of a suitable rinse liquid to the channel 20 and spinning of the disc to move the rinse liquid outwardly along channels 13 by centrifugal force.

**[0102]** Figure 8 shows a section of a CD, 23 having two consecutive inner annular hydrophilic channels, 24 and 25 which are connected by a radial hydrophilic channel 26 and a channel 27 which contains a hydrophobic area or break A. The outermost annular channel 25 is connected to an annular waste channel 28 by a radial hydrophilic overflow channel 29 having a hydrophobic break or valve Y2 adjacent to the junction with the waste channel 28. The annular channel 25 is also connected to two serially arranged chambers 30 and 31, the second of which is in turn connected to the waste channel 28. The annular channels 25 and 28 and the chambers 30 and 31 are connected via channels which contain hydrophobic breaks or valves B, C and D.

**[0103]** The innermost chamber 30 has a treated surface permitting the growth of cells within the chamber. It is also provided with an air channel 32, which contains a hydrophobic break, and which, alternatively, can act as a sample inlet port. The outermost chamber 31 has an untreated hydrophilic surface and can conveniently act as an analysis zone in conjunction with a detector (not shown).

**[0104]** Aqueous reagent for use in carrying out tests is introduced into annular channel 25 and feeds by capillary action into the radial channels until it reaches the hydrophobic breaks or valves B and Y2. The CD is then spun at a first rotation speed so that liquid passes through Y2 into the waste channel 28 and then through B until it reaches C. Cells are allowed to grow in chamber 30 and when cell culture has reached the required level the disc is spun again at a second, higher rotation speed so that the contents of chamber 30 are transferred into chamber 31, but prevented from travelling further by the hydrophobic breaks or valves D. An analysis, or further manipulation, can then be carried out in chamber 31 after which the CD is spun at a third

still higher, rotation speed so that the content of chamber 31 passes across D into the waste channel 28.

**[0105]** A rinse solution can then be introduced into the annular channel 24. The CD is spun again so that the solution passes through the hydrophilic breaks or valves Y and A, into the chambers 30 and 31 and then into the waste channel.

**[0106]** In order to prevent capillary "creep" of liquids around hydrophilic corners, a hydrophobic surface was applied to one side of the capillary channels, designated V in figure 8. (The channels are normally of square or rectangular cross section. The hydrophobicity and dimensions of the breaks or valves Y, Y2, A, B, C and D are chosen such that the force required to make liquid flow over D is greater than C which in turn is greater than B which is greater than Y2).

### **The Sample**

**[0107]** The sample applied to an inlet port may contain one or more analytes, which may comprise lipid, carbohydrate, nucleic acid and/or peptide structure or any other inorganic or organic structure. The sample treatment protocol to take place within the microchannel structure typically means that the sample is transformed to one or more MS-samples in which

**[0108]** (a) the MS-analyte is a derivative of the starting analyte and/or

**[0109]** (b) the amount(s) of non-analyte species have been changed compared to the starting sample, and/or

**[0110]** (c) the relative occurrence of different MS-analytes in a sample is changed compared to the starting sample, and/or

**[0111]** (d) the concentration of an MS-analyte is changed relative the corresponding starting analyte in the starting sample, and/or

**[0112]** (e) sample constituents, such as solvents, have been changed and/or the analyte has been changed from a dissolved form to a solid form, for instance in a co-crystallised form.

[0113] Item (a) includes digestion into fragments of various sizes and/or chemical derivatization of an analyte. Digestion may be purely chemical or enzymatic. Derivatization includes so-called mass tagging of either the starting analyte or of a fragment or other derivative formed during a sample treatment protocol, which takes place in the microchannel structure. Items (b) and/or (c) include that the sample analyte has been purified and/or concentrated. Items (a)–(d), in particular, apply to analytes that are biopolymers comprising carbohydrate, nucleic acid and/or peptide structure.

[0114] The sample is typically in liquid form and may be aqueous.

[0115] The sample may also pass through the microchannel structure without being changed. In this case the structure only provide a proper form for dosing of the analyte to the mass spectrometer.

#### **The microfluidic structure**

[0116] The microfluidic device comprises one or more microchannel structures having an inlet port for application of a liquid sample and an MS-port for release and presentation of an MS-analyte to a mass spectrometer. These kinds of ports may coincide in a microchannel structure. There may also be separate inlet ports for application of solvents and reagents and separate outlet ports or waste chambers/cavities for withdrawal of other components that are added and/or produced in the structure. Two or more microchannel structures may have common inlet ports. Depending on the particular design of the device some of the ports may be closed during the sample treatment but opened later on, for instance in order to enable proper release and presentation of the MS-analyte.

[0117] The distance between two opposite walls in a channel is typically  $\leq 1000\ \mu\text{m}$ , such as  $\leq 100\ \mu\text{m}$ , or even  $\leq 10\ \mu\text{m}$ , such as  $\leq 1\ \mu\text{m}$ . Functional channel parts (chambers, cavities etc) typically have volumes that are  $\leq 500\ \mu\text{l}$ , such as  $\leq 100\ \mu\text{l}$  and even  $\leq 10\ \mu\text{l}$  such as  $\leq 1\ \mu\text{l}$ . In important variants these volumes may be  $\leq 500\ \text{nl}$  such as  $\leq 100\ \text{nl}$  or  $\leq 50\ \text{nl}$ . The depths of these parts may be in the interval  $\leq 1000\ \mu\text{m}$  such as  $\leq 100\ \mu\text{m}$  such as  $\leq 10\ \mu\text{m}$  or even  $\leq 1\ \mu\text{m}$ . The lower limits (width and depth) are always significantly greater than the largest of the reagents and analytes (including fragments and derivatives) that are to be transported within the microchannel structure. The lower limits of the different channel parts are typically in

the range 0.1-0.01  $\mu\text{m}$ . The aspect ratio (depth to width) may be  $\geq 1$  or  $\leq 1$  in all parts or in only a part of a microchannel structure.

**[0118]** Preferred microfluidic devices typically comprise one, two or more, preferably more than 5, microchannel structures fabricated wholly or partly in the surface of a planar substrate. In the preferred microfluidic devices of the invention, the side of the substrate in which the microchannels are located (microchannel side) may be covered by a lid comprising remaining parts, if any, of the microchannel structure. When the lid is properly mated to the upper side of the substrate, parts of the microchannel structures in the lid, if any, match the structures in the microchannel side thereby completing the microchannel structures of the device. The lid will prevent or minimise undesired evaporation of liquids as well as facilitate transport of liquids.

**[0119]** Each microchannel structure preferably extends in a common plane of the planar substrate material. In addition there may be extensions in other directions, primarily perpendicular to the common plane. Such other extensions may function as sample or liquid application areas or connections to other microchannel structures that are not located in the common plane, for instance.

**[0120]** The microfluidic devices may be disc-formed and have various geometries, with the circular form being the preferred variant (CD-form). Other variants of discs may have an axis of symmetry that is at least 3- or at least 6-numbered.

**[0121]** On devices having circular forms or an axis of symmetry as mentioned in the previous paragraph, each microchannel structures may be oriented radially around a central axis with an intended flow direction for each structure from an inner application area (inlet port) towards the periphery of the disc. The arrangement may be in form of one or more concentric circles (annular/circular arrangements). According to the first aspect of the invention the liquid is transported by inertia force, for instance centrifugal force, in at least a part of a microchannel structure. Examples of other ways of transportation are by capillary action, hydrodynamically, electrokinetically etc. These alternatives may also be combined with inertia force in line with what has been discussed for an aspect of the invention.

**[0122]** Each microchannel structure comprises one or more channels in the microformat. The channels may comprise chambers/cavities that are in the microformat.

Different parts of a structure may have different discrete functions. In addition to the channel parts mentioned above (inlet port, MS-port, transportation conduit/channel), there may be one or more channel parts that function as

[0123] (a) application zone/port for reagents and liquids other than sample liquid (second inlet port),

[0124] (b) additional MS-ports,

[0125] (c) reaction zone, for instance for derivatization of an analyte discussed above (digestion, tagging etc).

[0126] (d) pressure creating zone (for instance hydrostatic pressure),

[0127] (e) volume defining zone,

[0128] (f) mixing zone,

[0129] (g) zone for separating and/or concentrating and/or purifying the analyte or a derivative or fragment thereof, for instance by capillary electrophoresis, chromatography and the like,

[0130] (h) waste conduit/chamber/ cavity (for instance in the form of an outlet port),

[0131] (i) zone for splitting a liquid flow, etc.

[0132] These kinds of zones may be present as distinct chambers or conduits that may have a cross-sectional dimension that differs from a preceding and/or a subsequent part of the microchannel structure.

[0133] Splitting may be located at the inlet so that a starting sample is divided in several aliquots, each of which is processed in parallel within a structure.

[0134] Except for the presence of an MS-port this kind of microchannel structures have been described in a number of previous patent publications. See the background publications discussed above.

[0135] Between parts having different functions there may be valves that can be overcome by increasing the force driving the liquid. For variants utilizing spinning, this may for instance be accomplished by increasing the spinning and/or utilizing pressure built up within the structure due to addition of a new portion of liquid combined with spinning. See for instance WO 0040750 (Gyros AB) and WO 0146465 (Gyros AB). Valves may be based on capillary junctions (WO 9807019 (Gamera Bioscience)) or hydrophobic breaks (WO 9958245 (Gyros AB) or on thermic properties of the valve material. The latter kind of valves may be illustrated by so called sacrificing valves (WO 9853311 (Gamera Bioscience)) for instance containing a plug of wax-like material, or reversible valves, for instance containing a thermoreversible polymer in the form of a plug (WO 0102737 (Gyros AB)).

[0136] One kind of versatile microchannel structures used according to the invention comprises a zone in which separation and/or concentration and/or a purification of the analyte or an analyte-derived entity can take place. This zone is located either before or in the MS-port. Examples of analyte-derived entities are fragments and derivatives of the analyte. This kind of functionality may be particularly important for samples containing low concentrations of analytes, complex mixtures of analytes or high concentrations of interfering substances that may negatively affect the resolution and/or sensitivity of the MS-analyte when analyzed by mass spectrometry. Separation and/or concentration and/or purification of the sample analyte or analyte-derived entities can be accomplished according to similar principles as typically employed in the life science area, i.e. separations based on size exclusion and/or on differences in binding to a ligand structure are applicable. Accordingly, this kind of channel part may contain a separation medium that is capable of binding the analyte or an analyte-derived entity but not to the contaminants, or vice versa. The separation medium is typically in particle/bead form, or attached on the surface of the separation zone or in the form of a monolithic plug that permits through flow. If the analyte or the analyte-derived entity becomes bound, a liquid having the proper desorption characteristics for the bound entity is subsequently allowed to pass through the chamber whereupon the bound entity is released and transported downstream. This transport may be directly to the MS-port or to a zone in which a further preparation step is accomplished. Washing steps may be inserted between the sample liquid and the desorption liquid. The separation medium may be soluble or insoluble during the binding step. Soluble separation media are typically insolubilized after binding according to principles well-known in the field of macroscopic separations.

**[0137]** Binding to the separation medium may involve formation of covalent bonds or encompass affinity binding. Binding of covalent nature for this purpose is typically reversible, for instance by thiol-disulfide exchange, such as between a thiol-containing analyte or analyte-derived entity and a separation medium containing a so called reactive disulfide, or vice versa. Affinity binding, including adsorption, can be illustrated with:

**[0138]** (a) electrostatic interaction that typically requires that the ligand and the entity to be bound have opposite charges,

**[0139]** (b) hydrophobic interaction that typically requires that the ligand and the entity to be bound comprises hydrophobic groups,

**[0140]** (c) electron-donor acceptor interaction that typically requires that the ligand and the entity to be bound have an electron-acceptor and electron-donor group, respectively, or vice versa, and

**[0141]** (d) bioaffinity binding including other kinds of binding in which the interaction is of complex nature, typically involving a mixture of several different kinds of interactions and/or groups.

**[0142]** Ion exchange ligands may be cationic (= anion exchange ligands) or anionic (= cation exchange ligands). Typical anion exchange ligands have a positively charged nitrogen, the most common ones being primary, secondary, tertiary or quaternary ammonium ligands, and also certain amidinium groups. Typical cation exchange ligands are negatively charged carboxylate groups, phosphate groups, phosphonate groups, sulphate groups and sulphonate groups.

**[0143]** Bioaffinity binding includes that the analyte or the analyte-derived entity is a member of a so-called bioaffinity pair and the ligand is the other member of the pair. Typical bioaffinity pairs are antigen/hapten and an antibody/antigen binding fragment of the antibody; complementary nucleic acids; immunoglobulin-binding protein and immunoglobulin (for instance IgG or an Fc-part thereof and protein A or G), lectin and the corresponding carbohydrate, etc. The term “bioaffinity pair” includes affinity pairs in which one or both of the members are synthetic, for instance mimicking a native member of a bioaffinity pair.



[0144] If the analytes in a sample have peptide structure or nucleic acid structure or in other ways have a pronounced hydrophobicity, the separation medium may be of the reverse phase type (hydrophobic) combined with using desorption liquids (eluent) that are organic, for instance acetonitrile, isopropanol, methanol, and the like. Depending on the particular sample and the presence of analytes or analyte-derived entities, which have a common binding structure, a group-specific separation medium may be utilized for the kind of separations discussed above. The separation medium may thus, like a reverse phase adsorbent, result in an MS-sample that has a reduced concentration of salt, i.e. in desalting.

[0145] In each microchannel structure there may be two or more separations zone. In this case the zones typically separate according to different principles such as size and charge. For amphoteric substances such as proteins and peptides the latter principle may be illustrated with isoelectric focusing.

[0146] By using a separation zone it is possible to concentrate the sample such that the concentration of an analyte or an analyte-derived entity in the desorption liquid after passage of the separation medium is higher than in the starting sample. The increase may be with a factor  $> 100$  and may typically be found in the interval  $10^1$ - $10^6$ , such as  $10^1$ - $10^4$ .

[0147] As already mentioned a separation zone may be combined with zones for derivatization. There may also be microchannel structures that have a derivatization zone but no separation zone.

[0148] Figure 9 illustrates a microchannel structure that comprises (a) an inlet port (1) for liquids including the sample liquid, (b) an MS-port (2) comprising for instance an EDI area that may be opened or closed, (c) a flow conduit (3) between the inlet port (1) and the MS-port (2). The flow conduit (3) may have a zone (4) containing an adsorbent for separation/concentration. If there are several microchannel structures in a device there may be a common application area/channel with openings for the inlet ports (not shown). The MS-port may be an EDI MS-port, an electrospray MS-port.

[0149] The use of the structure of figure 9 is as indicated. By using spinning for liquid transportation into an open form of the MS-port, aqueous liquids, for instance the sample or the washing liquids, may leave the port as small drops by the centrifugal force while liquids

having a lower vapour pressure may evaporate leaving the MS-analyte in the port, e.g. at a lower spinning rate.

**[0150]** Figure 10 illustrates another variant of a suitable microchannel structure. It has two inlet ports (5,6) that may be used for application of sample, washing liquids and desorption liquid. One of the inlet ports (5) is connected to an application area/channel (7) that may be common to several microchannel structures in the same device. This first inlet port (5) is connected to one of the shanks (8) in a U-shaped channel part via the application area/channel (7). The other inlet port (6) is connected to the other shank of the U. In the lower part of the U there is an exit conduit (9) leading to an MS-port (10). In the channel (11) between the exit conduit (9) from the U and the MS-port (10) there may be a zone (12) containing a separation medium. From the MS-port (10) there may be a waste channel (13) leading to a waste space (14) that may be common for several microchannel structures in the same device. There may be a valve function, for instance in the form of a hydrophobic break, in the exit conduit (9).

**[0151]** Figure 11 illustrates another alternative of a microchannel structure which comprises a separate sample inlet port (14), an MS-port (15) and therebetween a microchannel structure that may be used for sample preparation. In this variant there is a volume-defining unit (16) between the two ports (14,15) with an over-flow conduit (17). At the lower part of the volume-defining unit (16) there is a first exit conduit (18) leading to one of the shanks (19) of a U-shaped channel part. The other shank (20) of this U may be connected to an inlet port (21) for washing and desorption liquids. At the lower part of the U-shaped channel part there may be a second exit conduit (22) leading into one of the shanks (23) of a second U-shaped channel part. The other shank (24) may be connected to a waste channel (25b) that after a bent (26) may end in a waste chamber (25a). At the lower part of this second U-formed channel part there may be a third exit conduit (27) leading into the MS-port (15) that may contain an EDI area or an electrospray unit. In order to control the flow in the structure, valve functions are preferably located in the first exit conduit (18), for instance immediately downstream the volume-defining unit (16), possibly also in the second exit conduit (22), for instance immediately after the first U-shape, and in the third exit conduit (27), for instance immediately after the second U-shaped channel part. The valves may be of the types discussed above with preference for hydrophobic breaks. A suitable adsorbent (28) as discussed above may be placed in the shank (23) of the second U-shaped channel part and may also function as a valve. In case the adsorbent is in the

form of particles they are preferably kept in place by a constriction of the inner walls of the conduits.

**[0152]** The structure presented in figure 11 is adapted for transporting the liquid with centrifugal forces, i.e. with the structure present in a disc and oriented radially outwards from the centre of the disc. At start the volume-defining unit (16) is filled up somewhat above the over-flow channel (17). By overcoming a valve function located in the first exit conduit (18), the liquid will pass into the first U-shaped channel part and down through the adsorbent where the analytes are captured. The remaining liquid containing non-analyte components will pass out into the waste channel (25b). In the next step, washing liquids may be applied through the inlet port (21), i.e. through the second shank (20) of the first U-shaped channel part or via the same inlet port (14) as the sample. Also these liquids will pass out into the waste channel (25b). Subsequently, a desorption liquid is applied through either of the two inlet port (14,21) and allowed to pass through the valve function in the third exit conduit (27). The desorption liquid containing released analyte or analyte-derived entities is passed downstream, for instance into the MS-port (15). The operations are preferably carried out while spinning the disc. If the valves are in the form of hydrophobic breaks they can be passed by properly adapting the g-forces, i.e. by the spinning. By properly balancing the hydrophilicity/hydrophobicity of a liquid, passage or non-passage through a valve may be controlled without changing the spinning speed. This is illustrated by utilizing a hydrophobic break as the valve in the third exit conduit (27) combined with utilizing water-solutions as samples and as washing liquids and liquids containing organic solvents as desorption liquids. In the alternative, valves that are opened by external means can be used. By placing the outlet to the first exit conduit (18) at a distance above the lowest part of the volume-defining unit (16) particulate matters, if present in the sample, will sediment and be retained in the volume-defining unit (16) when it is emptied through the first exit conduit (18).

**[0153]** Calibrator areas (29) are shown in each of figures 9-11. Each calibrator area may be connected to a common area for application of calibrator.

**[0154]** The size of a volume-defining unit depends on the sample, reagents used, washing etc. and the requirement the sensitivity of the mass spectrometer sets for concentrating an analyte or an analyte-derived entity. Typical volumes of channel parts that have specific functions are in the range of 1 nl to 1000  $\mu$ l, mostly below 1  $\mu$ l such as below 500 nl or even below 100 nl such as below 25 or 10 nl (volume defining unit, reactor part, separation part etc).

Application of aliquots of a sample to the same inlet port may replace the need for a larger volume defining unit.

**[0155]** These kind of flow systems has been described in WO 0040750 (Gyros AB) and WO 0146465 (Gyros AB) which are hereby incorporated by reference.

**[0156]** In certain variants the inlet port for the sample and the MS-port may coincide. In this case the MS-port preferably comprises the surface on which the analyte can be collected (adsorbed). Remaining liquid and washing liquids, if used, are passed into the microchannel structure that then will function as a waste channel and possibly contain a separate outlet port particularly adapted for wastes and the like, or a waste chamber. In order to accomplish a concentrating and/or separating effect the surface may expose structures selectively binding/capturing the analyte as discussed above. This variant also encompasses that there may be a separate inlet port for washing and desorption liquids and microchannel part communicating with the combined sample and MS-port.

#### **The MS-port**

**[0157]** The invention the MS-port typically has conducting properties. The conducting part may be present in an EDI area or a nozzle suitable for electrospray, for instance a nanospray, or in any other form device that is used to present a sample to a mass spectrometer. An electrospray nozzle provides an orifice for instance in the form of a tip with a through-passing hole. Various kinds of sample presentation devices have been described in the publications discussed above.

**[0158]** There may be a valve in the microchannel before its inlet to the MS-port.

**[0159]** The term conducting material includes semi-conducting material, although materials having a conductivity that is larger than silicon or larger than germanium are preferred. A typical conducting material comprise

**[0160]** (a) metals such as copper, gold, platinum etc,

**[0161]** (b) conducting polymers including polymers that are conducting as such, and conducting composites containing a non-conducting polymer and a conducting material such as a metal or a conducting polymer,

[0162] (c) other conducting composites, etc.

#### **Electrospray ionisation MS-ports**

[0163] Figure 12 illustrates an MS-port suitable for electrospray ionisation in a mass spectrometer. This kind of port may be located where an MS-port has been indicated in any of the structures given in figures 9-11. The MS-analyte may thus be collected in an MS-port comprising a collection zone (30), which zone is in fluid communication via the electrospray conduit (31) with the outlet orifice (32). The electrospray conduit may be in the form of a tip. The MS-analyte is entering via conduit (32). As indicated in figure 12, orifices of the electrospray tip are preferably positioned on the edge of a disc. Typical disc-forms have been discussed above. In use an electrospray orifice is matched to the sampling orifice of a mass spectrometer and liquid in the electrospray tip (31) is sprayed into the mass spectrometer. In a preferred variant the disc is circular. The arrangement of the electrospray tips is preferably annular around the axis for spinning during preparation of the sample. The tips are preferably located in the edge of the disc and provide a radial spray direction. In the alternative the annular arrangement may be in a flat side of the disc with a spray direction out of the plane of the side and preferably also with a component that is directed radially. Annular arrangements preferably in the edge of a circular disc will simplify accurate positioning of the electrospray direction relative to the sample application opening of a mass spectrometer.

[0164] Electrospray suitable for electrospray ionization mass spectrometry (ESI MS), for instance adapted to the nanospray format, is mostly formed in capillaries made of glass or fused silica, or polymer material like silicon. The tubings are typically of cylindrical geometry with tip internal diameters in the 5-20  $\mu\text{m}$  range. The word nanospray means that the liquid transferred out of the tubing is in the nanoliter per minute range. Suitable rates for transfer of liquid to the mass spectrometer can be found in the interval of 1-1000 nl/min, e.g. in the interval 10-500 nl/min. By infusion (no external force), only a few nanoliters per minute (5-25 nl/min) is transported out of the tubing while with applied external pressure 50-500 nl/min is more common.

[0165] A liquid solution suitable for ESI MS analysis consist of an organic solvent:water mixture including a lower concentration of acid or base. The composition of the solvent is important especially with regard to surface tension and conductivity. A low surface tension and conductivity is desirable to obtain an efficient desolvation and ionization process as

well as a stable spray. If the sample is dissolved in water only, a so-called make-up solvent is preferably added (external delivery) to aid in the above mentioned process. A make-up solvent is typically configured co-axially (sheat-flow) around the nanospray tip. A make-up gas (typical N<sub>2</sub>) is sometimes added (e.g., co-axially) as to aid in the desolvation process. Creation of a suitable liquid composition of the MS-sample may also be part of the sample preparation process taking place upstream the MS port in other parts of a microchannel structure.

**[0166]** The tip geometry is important as to obtain a stable spray. Preferably the tip is pulled from the cylindrical tubing whereby the tip becomes oblong with a conical shape. The outer diameter of the tubing near the orifice of the tip then becomes of similar dimension as the internal diameter.

**[0167]** In order to induce a spray from the capillary (towards the inlet of the mass spectrometer) a voltage has to be applied on the electrospray tip. Therefore the tip has to be made conductive. Different kind of metals can be deposited by different techniques onto the tip (or part of the tubing). Important aspects here regard the stability (life-time) of the metallized tip since the voltage applied as well as different solvents affects its stability. Other possibilities also exist than metalization, e.g., an electrode can be inserted into the tip whereby a voltage can be applied to induce electrospray. Another alternative is to make the tip in a material comprising any of the above-mentioned conducting materials. Typical voltages used in nanospray range between 500-2000 volts.

**[0168]** Typical electrospray nozzles are available from a number of manufacturers, for instance New Objective, MA, U.S.A. A variant that is believed to have advantages for microfluidic devices is presented in WO 0247913 (Gyros AB). See also WO 9704297 (Karger et al), US 5,969,353 (Hsieh) and US 6,110,343 (Ramsey et al) discussed above.

#### **EDI MS-ports**

**[0169]** The MS-port may also be used for EDI-MS and will then contain an EDI-area. Upward and downward directions as used in the context of EDI areas refer to the directions defined in the figures irrespective of how the area is positioned in a mass spectrometer.

**[0170]** The term “EDI area” encompasses the surface of the area as well as any part of the device, which is straight under the surface.

**[0171]** EDI MS ports may be adapted to anyone of the different EDI mass spectrometry variants, for instance Time of Flight (TOF), Quadropole, Fourier-Transformed Ion Cyclotron Resonance (FT-ICR), ion trap etc.

**[0172]** EDI ports need a free opening for the release of the ions created during desorption/ionisation. This means that the opening typically is placed straight above the surface of an EDI area and has an area that should be coaxial with and covering the surface. This includes that the opening may be closed during the sample treatment within the microfluidic device but subsequently opened to enable irradiation and desorption/ionisation and possibly also evaporation of solvents. If an IDI principle is used the opening should also provide space for the incident irradiation.

**[0173]** An EDI area may in principle have any geometric form although preferred forms should be as compact as possible. Regular forms, such as squares and square-like forms, and rounded forms, such as circular and circle-like forms. The size of an EDI area preferably is the same as a circle with a diameter in the interval of 25-2000  $\mu\text{m}$ . For IDI there may be advantages if the cross-sectional area of the incident beam is able to encompass the complete surface of an IDI area or as much as possible, for instance more than 25 % or more than 50 %.

**[0174]** An EDI area comprises a conducting layer (layer I), for instance a metal layer of copper, gold, platinum, stainless steel etc or a layer of any other conducting material of the kinds discussed above. Layer (I) is many times essentially planar and may coincide with the surface of the EDI area or be parallel thereto. The complete area from the lowest part to the most upper part may be made of conducting material, i.e. correspond to layer (I). In the case the microfluidic device comprises more than one microchannel structure with an EDI MS-port, layer (I) of one EDI area may extend into and encompass layer (I) in two or more of the other EDI areas, with preference for all of them. Layer (I) of all EDI areas of a device may be part of a common conducting layer, which may be essentially planar and possibly with depressions corresponding to the EDI areas and/or to other parts of the microchannel structures of the innovative device. The exact geometric shape of layer (I) outside the MS-port may differ depending on the particular device and practical ways of its manufacture. For instance a common conducting layer may have an annular form in case the MS-ports are annularly arranged, i.e. only be present in EDI areas of a certain distance from a the centre of the arrangement.

**[0175]** In one innovative variant, there is a non-conducting layer (layer (II)), which covers the conducting layer (I). Layer (II) in one EDI area may extend into and encompass layer (I) in two or more of the other EDI areas as described for layer (I).

**[0176]** In another innovative variant the device have a conducting layer (III) positioned above the common plane defined by the surface of each EDI area of a device. Layer (III) has openings matching the surfaces of the EDI areas and permitting irradiation of the EDI surfaces and escape of ions produced through the openings.

**[0177]** These innovative variants of EDI areas are schematically illustrated in figures 13a-f, each of which shows a cross-sectional view across the EDI areas of two EDI MS-ports in a microfluidic device according to the invention. The EDI surfaces are referenced as (51) and the EDI areas as (52) within the dotted vertical lines). Each EDI port comprises the EDI area plus the corresponding depression. The conducting layers (54) are hatched. It is apparent that each EDI area comprises a conducting layer (I) (53).

**[0178]** Figure 13a shows a common conducting layer (54) at the bottom of the device which layer encompasses layer (I) (53) of each EDI area (52). A non-conducting layer (II) (55) is placed between layer (I) (53) and the EDI surface (51). Figure 13b shows a variant, which is similar to the variant in figure 13a, but the common conducting layer is embedded within the material from which the device is fabricated. Layer (II) (55) is present. In figure 13c there is a common conducting layer (54) comprising the upper surfaces of the EDI areas. In figure 13d there is no common conducting layer. Layer (I) is in the upper surfaces of the EDI areas. The different layers (I) (53) are isolated from each other. Figures 13e shows a variant in which there is a continuous conducting layer (54) above layer (I) (53) of the EDI areas. This conducting layer (54) has openings (56) corresponding to the openings of each MS-port and may be the surface of the upper or lower side of a lid covering the microchannel structure (the lid is not shown). Figure 13f shows a variant in which there is a common continuous conductive layer comprising layer (I). Layer (I) corresponds to the EDI surfaces. The continuous layer also encompasses the walls of the depressions in the EDI ports.

**[0179]** In one variant all the material of the body in which the microchannel structures are fabricated is the same as in layer (I), i.e. there may be no non-conducting material present, except for the lid.



**[0180]** Figure 14 illustrates an arrangement for EDI MS-ports on a circular disc (with a transparent lid), in which layer (I) (34) of each EDI surface has a conductive connection (35) with a peripheral conductive layer (36) at the edge of the disc. In the variant shown in this figure each microchannel structure (37) extends upstream to an inlet port (38).

**[0181]** Figures 15a-b illustrate an EDI MS-port which the opening to the EDI surface is defined by a hole (39) in a lid (40) which in this case is transparent. One can see the incoming microchannel (41), which opens to a circular depression (42) with a diameter, which is less than the diameter of the hole (39). Layer (I) (43), EDI area (44, between the dotted lines), EDI surface (45) are shown. The conductive layer extends from layer (I). This design in which the MS-port provides an opening which is greater than the EDI area will facilitate for an incident beam to cover any spot of the EDI surface.

**[0182]** A conductive layer per se may function as a conductive connection or there may be distinct connections (35) to layer (I). See figure 14.

**[0183]** A lid used to cover the microchannel structure may also cover the EDI areas or restrict them from exposure to the ambient atmosphere. In this case there may be advantages if the lid is removable at least around the EDI areas. Thus after treatment in upstream parts of the microchannel structure, the EDI areas can be exposed for evaporation of solvents that may be retained thereon or for irradiation in order to accomplish desorption/ionisation of MS-analyte molecules.

**The method steps of an aspect of the invention.**

**[0184]** When the innovative device is used, the necessary reagents and/or the sample including the analyte are applied to the appropriate inlet ports (application areas) and transported downstream in the structure by an applied liquid flow or transportation mechanism as discussed above. The liquid flow or transport may be paused when a reagent and/or an analyte have reached a predetermined part in which the sample is subjected to a certain procedure, for instance capillary electrophoresis in a separation part, a chemical reaction in a reaction part, selective adsorption in a concentrating part, detection in a detection part, evaporation and/or crystallization in an EDI MS-port, collection of the appropriate liquid for electrospray ionisation in an electrospray ionisation MS-port, etc.

**[0185]** During transport through a microchannel structure the solvent composition may be changed to fit the particular kind of MS-port used. In the case the MS-port is an EDI MS-port and the microchannel structure contains a separation medium, an EDI matrix may be (a) included in the desorption liquid or in a subsequent liquid passing through at least a part of the microchannel structure, (b) predisposed to the EDI surface or dispensed after the analyte has been deposited on the surface, or (c) introduced via a separate microchannel downstream the separation medium. There may also be included substances that facilitate crystallization on the EDI surface.

**Calibration of the mass scale.**

**[0186]** To ensure accurate mass determination, calibrator areas (spots) containing a compound of known molecular weight (standard) may present in the proximity of the EDI MS-ports. Calibrator areas (29) are shown in figures 9-11. Alternatively, the standards may be included in the sample or added to the EDI area before desorption/ionisation (internal calibrator). The choice of calibrator substance, its amount etc will depend on its use as an external or internal calibrator, the MS-analyte and its concentration etc.

**Material from which the microfluidic device is manufactured.**

**[0187]** The microchannel structures are typically fabricated in inorganic and/or organic material, preferably plastics or other organic polymers. The material may be conducting or non-conducting as already discussed. Certain parts of a microchannel structure may be metalized.

**[0188]** Suitable organic polymers may derive from polymerisation of monomers comprising unsaturation such as carbon-carbon double bonds and/or carbon-carbon-triple bonds. The monomers may, for instance, be selected from mono-, di and poly/oligo-unsaturated compounds, e.g. vinyl compounds and other compounds containing unsaturation.

**[0189]** Another type of organic polymers that may fit are based on condensation polymers in which the monomers are selected from compounds exhibiting two or more groups selected among amino, hydroxy, carboxy etc groups. The plastics contemplated are typically polycarbonates, polyamides, polyamines, polyethers etc. Polyethers include the corresponding silicon analogues, such as silicone rubber.

**[0190]** The polymers of the plastics are preferably in cross-linked form.

[0191] The plastics may be a mixture of two or more different polymer(s)/copolymer(s).

[0192] At least a part of the microchannel structure may have a surface that has been derivatised and/or hydrophilized, for instance by being coated with a non-ionic hydrophilic polymer according to the principles outlined in International Patent Publication WO 0147637 (Gyros AB). This also includes functionalisation in order to introduce one or more structures that are capable of interacting with the sample analyte or with one or more of the reagents added. Surfaces may be made of copper, gold, platinum, stainless less etc, for instance by metallization, in order to enable a desired derivatization or for providing a conducting surface, for instance in an MS-port. Gold surfaces for instance may be derivatized by reaction with thiol-containing compounds that have a desired functionality, for instance hydrophilicity.

[0193] The optimal water contact angle for the surfaces within a structure depends on the protocols to be carried out, the dimensions of the microchannels and chambers, composition and surface tension of the liquids, etc. As a rule of thumb, at least one surface of the microchannel structure (wall, bottom or top), should provide a water contact angle that is  $\leq 40^\circ$  or  $\leq 30^\circ$ , such as  $\leq 25^\circ$  or  $\leq 20^\circ$ . These figures refer to values obtained at the temperature of use, primarily room temperature.

[0194] It is believed that the preferred variants of the inventive microfluidic devices will be delivered to the customer in a dried state. The surfaces of the microchannel structures of the device therefore should have a hydrophilicity sufficient to permit the aqueous liquid to be used to penetrate the different parts of the channels of the structure by capillary forces (self-suction). This of course only applies if a valve function at the entrance of the particular part has been overcome.

#### **Best Mode for Mass Spectrometry**

[0195] The best mass spectrometric results so far accomplished have been with the variant described in example 4 below. It is believed that there are advantages by going over to more complex fluidic structures as illustrated in figures 10 and 11, and more complex designs of the EDI surface and the channel outlets as illustrated in figure 15.

[0196] The following examples illustrate the preparation of surfaces having different characteristics on a hydrophobic substrate.

### EXAMPLE 1

[0197] A CD disc made from Zeonex ( a cycloolefin copolymer manufactured by is Nippon Zeon), having recessed microfabricated channels on the surface, was masked selectively by applying a viscous film-forming fluid at desired spots in the channels. As the film-forming fluid was used either Owoco Rod (based on a synthetic water-soluble polymer) or Owoco Rosa (based on a synthetic rubber latex dispersion), both delivered by Owoco AB, Stockholm, Sweden. After drying, the disc was placed in a plasma reactor (Plasma Science PS0500 from BOC Coating Technology, Concord Ca USA) and treated with an oxygen plasma (5 cm<sup>3</sup>/min gas flow, 500 W RIF power) for 10 min. The mask was then removed by water rinsing followed by an ethanol rinse. The non-masked areas had a water contact angle of 5 degrees, while the masked areas had a contact angle of 90 degrees. A soft silicone rubber lid was placed over the disc and an aqueous dye solution was introduced in the channels. The solution penetrated by self- suction into the non-masked channel areas, but stopped at the hydrophobic masked areas. By spinning the disc at 3000 rpm, the solution could be made to pass also over the masked areas.

### EXAMPLE 2

[0198] A CD disk made from polycarbonate, having recessed microfabricated channels on the surface, was placed in a plasma reactor (Plasma Science s PS0500 from BOC Coating Technology, Concord Ca USA) and treated with an oxygen plasma (5 cm<sup>3</sup>/min gas flow, 500 W RF power) for 10 min. After treatment the disc surface had a water contact angle of 5 degrees. A 0.5% solution of polyisobutylene in cyclohexane was then applied locally at selected spots and left to dry in. The polyisobutylene-coated areas had a water contact angle of 100 degrees. A soft silicone rubber lid was then placed over the disc and an aqueous dye solution was introduced in the channels. The solution penetrated by self-suction into the non-coated channel areas, but stopped at the hydrophobic coated areas. By spinning the disc at 3000 rpm, the solution could be made to pass also over the coated areas.

### EXAMPLE 3

[0199] A C.D. disk made from polycarbonate, having recessed microfabricated channels on the surface, was patterned with gold by evaporation through a shadow mask. First a 40nm think layer of chromium was evaporated through the mask. The CD disc was then placed in a plasma reactor (Plasma Science PS0500 from BOC Coating Technology, Concord Ca USA)

and treated with an air plasma (10 cm<sup>3</sup>/min gas flow, 500 W RF power) for 10 min. After treatment the disc surface had a water contact angle of 6 degrees. The CD disc was then placed in glass container and 50 ml of a 1 mM solution of octadecylmercaptane in ethanol was added. After one hour in the thiol solution, the CD disc was carefully rinsed by ethanol. The water contact angle on the polycarbonate area was 7 degrees, and 79 degrees on the gold surface. A soft silicone rubber lid was then placed over the disc and an aqueous dye solution was introduced in the channels. The solution penetrated by self-suction into the non-coated channel areas, but stopped at the hydrophobic gold-coated areas. By spinning the disc at 3200 rpm, the solution could be made to pass also over the coated areas.

Table 1

Surface	Water contact angle (degrees)
Polytetrafluoro-ethylene (Teflon)*	108
Polyethylene*	94
Polypropylene*	95
Polymethyl methacrylate*	80
Platinum*	40
Glass**	"small"
Gold*	65.5

\*A.C. Zettlemoyer (Hydrophobic surfaces, Ed P M Fowkes, Academic s Press (New York) 1969, p.1-27

\*\* A.W. Adamson Physical chemistry of surfaces 5t" ed, Wiley- Interscience 1990, 9 397

#### EXAMPLE 4

##### Gold at different positions in a CD

Table 2

Gold patterning	Sensitivity*	Charging of substrate**
No gold	Poor	Yes
Gold on all sides	Good	No
Gold on upper side	Good	No
Gold on bottom side	Good	Yes
Isolated gold spots on the upper side	Good	Yes
Gold spots on the upper side. Every	Good	No

spot being conductively connected contact with the adapter plate through an individual gold string or a common gold area.		
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\* Good= sensitivity for a in-solution tryptic digest of BSA comparable to the sensitivity obtained on a conventional stainless steel target

\*\* Charging is observed as significant mass shift ( $\geq 1$  Da) upon repeated laser desorption/ionization and/or loss of signal.

[0200] Table 2 shows the results form a summary of experiments performed in order to optimise the design of the CD-MALDI interface. Gold was sputtered at various positions of the CD and the MALDI characteristics were studied for a tryptic digest of Bovine Serum Albumin (BSA). The CD was placed on a metal adapter inserted into the ion source. The gold was hence patterned in various ways to determine the importance of electrical contact between the MALDI ports and the adapter plate.

#### **EXAMPLE 5**

##### **Testing of crystallization on the MALDI surface**

[0201] Microfluidic device: The CD was fabricated of polycarbonate. Each microchannel structure in the CD only contained an inlet port (upstreams), the ms- port (downstreams) and a flow conduit between. The inlet and ms-port consisted of open areas made by drilling holes in the lid before covering the CD with the lid, i.e., the open areas on the CD was in contact with the surrounding atmosphere. Only a minor part of the flow conduit at the ms-port was in connection to the open area. The width and the depth of the microchannel leading into the ms-port were 100  $\mu\text{m}$  and 40  $\mu\text{m}$ , respectively. The open area of the ms-port had a cylindrical geometry with a diameter of 300  $\mu\text{m}$  (drilled hole) and depth 70  $\mu\text{m}$ , where the depth is the thickness of the lid. The bottom of the open area, therefore, constituted of the upper surface of the CD (and a minor part of the microfluidic channel) while the walls surrounding the open area, and restricting the dimension of the ms-port, was defined by the hole in the lid. The CD was metallized with gold (gold-sputtering) only on the side opposite to the microchannel structures.

[0202] Experimental: 100 nl of a sample consisting of two peptides, Angiotensin II (Mw 1046.2) and Bradykinin (Mw 1060.2), 20 fmol/ $\mu\text{l}$  each, dissolved in 50% isopropanol saturated with  $\alpha$ -cyanohydroxycinnamic acid (+ 0.1% trifluoroacetic acid) was applied in the

inlet hole by pipetting and transported to the open area by spinning (500 rpm). The CD was thereafter cut in half and the part containing the samples to be analysed was fixed by metal-screws onto a MALDI target probe adapted to contain on half of the CD. The probe was a Bruker standard target in the 96 well format. The CD + probe was then inserted into a Bruker Biflex MALDI time-of-flight (TOF) apparatus for mass spectrometry analysis.

[0203] Results: The sample liquid was slowly transported out into the open area (500 rpm). Once reaching the open area the solvent quickly evaporated and crystallization started. Crystals could be seen as dark spots in the open area of the ms-port. The volume of the ms-port was 5 nl while the sample applied was 100 nl. Since the volume transported out into the open area per time unit was less than 5 nl and solvent evaporation per time unit was faster than the incoming volume no overflowing in the ms-port was taking place. Therefore, the sample volume applied can far exceed the volume of the ms-port whereby the sample can be concentrated therein.

[0204] A high mass resolution was obtained since the peptide peaks were isotopically resolved. The peptide masses deviated slightly (less than 1 Dalton) from their theoretical values, but no calibration was performed. Also, the detection sensitivity has to be considered high since low femtomoles of peptides were detected (signal-over-noise ratio of 10) which is typically seen only in optimized MALDI TOF analysis.

#### **EXAMPLE 6**

##### **Planar CD and structured removable lid**

[0205] This example shows a planar CD in combination with a lid in which the microfluidic structures are present. The structured lid was achieved through casting Memosil (Hereaus, Germany) against a nickel-coated master. The microfluidic structure employed in this example is shown in figure 10.

[0206] The structured lid is attached to the CD by adhesion forces. The surface facing the lid should be hydrophilic as the presented invention utilizes capillary action to fill the microfluidic structures. This is especially important as the moulded lid, being a type of silicon rubber is hydrophobic.

[0207] The upper side of the CD was covered with gold using a DC Bias magnetron sputtering method ( $1 \times 10^{-5}$  torr, Ar plasma and titan as adhesion layer) and made

hydrophilic according to the following procedure. The gold sputtered side was cleaned by rinsing with ethanol, followed by an oxygen plasma treatment (Plasma Science PSO500,). After plasma cleaning a self-assembled monolayer (SAM) of hydroxylthiol was formed on the gold surface. The hydroxylthiol was 11-mercapto-1-undecanol (Aldrich, Milwaukee, WI) and used at a concentration of 2mM in degassed ethanol. To obtain a well-organized SAM, the gold sputtered disc was immersed in the thiol solution over night. After the thiol adsorption the CD was sonicated in ethanol for ca 2 min.

**[0208]** The lid, containing the microfluidic channels, was attached to the CD by adhesion forces. A second piece of polymeric material was mounted at a position of 180° from the structured lid as a counterbalance. Reversed phase beads (Source 15 RPC, Amersham Pharmacia Biotech, Sweden) with a diameter of 15 µm were packed into the individual structures using the filling port present in the common distribution channel. The slurry, containing the beads, was drawn into the individual channels by capillary action. Eighteen parallel reversed phase columns were formed when the disc was spun at 3000 rpm for 1 minute. The columns were rinsed with water containing 0.1% TFA (trifluoroacetic acid, Aldrich)) two times. The rinsing was performed at an rpm of 2500 for ca 1 min. 200 nL of in-solution tryptic digest of BSA was added to individual channels through the sample inlet. The following procedure was used for digestion. The BSA (Sigma) was dissolved to a final concentration of 4.75 pmol/µl in 0.1 ammoniumbicarbonate buffer at pH8. The enzyme-modified trypsin (Promega Corp., Madison, WI) was added and dissolved at a ratio of BSA/trypsin 20:1. The sample was incubated at 37° for 4 hours and then stored at -20 °C until used.

**[0209]** The sample was allowed to pass over the reversed phase columns at 1500 rpm. A second rinsing/washing step was performed as above using water containing 0.1% TFA. Finally the peptides were eluted using 200 nL eluent consisting 50% isopropanol, 50 % water and α-cyano-4-hydroxycinnamic acid (Aldrich) below saturation. The eluent was prepared by saturating a water:isopropanol (50%) mixture with α-cyano-4-hydroxycinnamic acid. To 100 µL of this mixture 200 µL of 50% water:50% isopropanol was added, resulting in an eluent saturated to approximately 2/3 with α-cyano-4-hydroxycinnamic acid.

**[0210]** The presentation of the sample in the MALDI port was performed in two different ways.



[0211] a) In the first example a full structure was utilised (figure 10). Eluent from the column was collected in the container placed at an outer radial position relative of the reversed phase column. When the lid was removed the liquid quickly evaporated leaving co-crystallized matrix and peptides on the gold sputtered surface. The disc was cut in halves to fit in the MALDI ionisation interface.

[0212] b) The moulded structure was cut directly after the packed column leaving an open-ended microstructure. The eluent was allowed to pass the column at a pre-determined speed (1500 rpm) in order to generate a controlled evaporation of the solvent at the MALDI port, and hence the formation of co-crystallized matrix and peptides suitable for MALDI analysis. The disc was cut in halves to fit in the MALDI ionisation interface.

#### **EXAMPLE 7**

##### **Structured CD and site-specific elution**

[0213] This example employs a CD with integrated microfluidic structures, a thin ( $\leq 70 \mu\text{m}$ ) lid with holes at positions matching the MALDI port in the CD. The microfluidic structure employed in this example is shown in figure 9.

[0214] The polycarbonate CD was covered with gold as described above. The side was hydrophilized using the thiolprocedure described above. The lid (SkultunaFlexible, Skultuna, Sweden), having, pre-drilled holes, was attached to the CD through heat pressing at  $135^{\circ}\text{C}$ .

[0215] Reversed phase beads (Source 15 RPC) with a diameter of  $15 \mu\text{m}$  were packed in the individual structures using capillary forces in combination with centrifugation. The columns were rinsed with ethanol and spun to dryness before 23 fmol of tryptically digested BSA was added and spun down using 700 rpm. The tryptic digest of BSA was generated according to the procedure described above. After sample addition, the column was rinsed twice with water.  $\alpha$ -cyano-4-hydroxycinnamic acid was mixed in an organic solvent of acetonitril/water 3:7 containing 0.1% TFA to a saturation of 2/3 and 250 nl was used to elute the sample from the 3 nl packed column.

[0216] The crystals obtained after evaporation of the organic/water mixture contained co-crystallized peptides. Eight singly charged peptide peaks were present in the mass spectrum obtained.